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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/763,076	05/14/2001	Ian Jeffrey Evans	109846.205/SYN-071	2695

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EXAMINER	
IBRAHIM, MEDINA AHMED	
ART UNIT	**PAPER NUMBER**
1638	11

DATE MAILED: 04/10/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/763,076	EVANS ET AL.	
	Examiner	Art Unit	
	Medina A Ibrahim	1638	

-- *The MAILING DATE of this communication appears on the cover sheet with the correspondence address* --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *30 January 2003* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-35* is/are pending in the application.

4a) Of the above claim(s) *13,17 and 22-35* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-12,14-16 and 18-21* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *12 April 2001* is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other:

DETAILED ACTION

Election/Restrictions

Applicant's election without traverse of Group I and SEQ ID NO: 3 in Paper No. 8 is acknowledged. Therefore, claims 1-12 and 14-16 and 18-21 are under examination. Claims 13 and 17, drawn to SEQ ID NOs: 4-7 and 25-28 and claims 22-35 are withdrawn from consideration as being drawn to a non-elected invention.

Sequence Listing

Applicant's CRF and paper sequence listing have been entered.

Drawings

The drawings filed with this application are approved by the Examiner.

Objections

In claim 2, "the said two or more protein" should be replaced with ---the two or more protein---.

Claims 5-9, 14 and dependents 10-13 and 15-17 are objected to under 37 CFR 1.75(c) as being in improper form because each depends upon another multiple dependent claim. See MPEP § 608.01(n). Accordingly, the claims 5-9, 14 and dependents 10-13 and 15-17 not been further treated on the merits.

Claim 11 is objected to for reciting non-elected inventions. Claims that recite non-elected inventions should be amended accordingly.

Claim Rejections - 35 USC § 112

1. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter, which the applicant regards as his invention.

2. Claims 1-12, 14-16 and 18-21 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1 and 3 are indefinite for lacking correlation between the preamble and the method steps. In claim 1, the preamble recites one or more proteins, and the body of the claim (line 3) recites two or more protein encoding regions.

Claims 1 and 3 are indefinite because "isolatable" in lines 5 and 6, respectively, implies that the propeptide may or may not be isolated from its source. It is suggested that "isolatable" be replaced with ---isolated---. Also, "derived" is indefinite because it is unclear what is encompassed in the derived product. Dependent claims 2 and 18-21 are included in the rejection.

In claims 3 and 15, "a fragment thereof" refers to?

In claims 6-8, "isolatable" should be replaced with ---isolated---, for clarification.

Claims 8 and 15 are indefinite because a "chimeric propeptide" is unclear and is not clearly defined in the specification.

In claim 9, a "hevein-type" antimicrobial protein is unclear. It is unclear how the "hevein-type" antimicrobial protein differs from the hevein one.

Claims 14-16 are indefinite for depending upon the non-elected claim 13.

Claims 18-19 are indefinite because the metes and bounds of a "protease-processing site" are unclear.

In claim 19, a "subtilisin-like" protease-processing site is unclear. It is unclear how the "subtilisin-like" protease processing site differs from the subtilisin one.

Claim 20, "derived" is indefinite as it is unclear what is encompassed in the derived product.

Claim 21 is indefinite because "multiple proteins" lacks antecedent basis in claim 1.

Claim Rejections - 35 USC § 112

3. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

4. Claims 1-12, 14-16 and 18-21 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of expressing two precursor plant defensins, the RsAFP2 and DmAMP1 separated by specific linker propeptides in a transgenic plant, does not reasonably provide enablement for a method that employs any fragment and variant of a linker propeptide from a plant antimicrobial protein and/or a virus to improve the expression levels of multiple proteins. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

The claims are broadly drawn to a method of improving expression of multiple proteins comprising inserting into the genome of said plant a DNA sequence comprising operably linked promoter region, a signal sequence, two or more protein encoding regions separated from each other by a linker propeptide coding sequence from plant antimicrobial proteins including DmAMP1 and Ac-AMP2 or from virus and fragments

and variants thereof, wherein the linker provides a post-translational cleavage site and wherein at least two of the protein encoding regions encode different proteins. The claims also encompass specific linker sequences, a hevein-type antimicrobial protein, and a subtilisin-like protease processing site.

Applicant teaches isolation of DmAMP1 cDNA and DmAMP1 gene from *Dahlia merckii* cDNA and genomic library prepared from near-dry seeds and leaves, respectively. The DmAMP1 gene encodes a precursor protein comprising a leader sequence with 28 amino acids, a mature protein with 50 amino acids, and a C-terminal propeptide with 40 amino acids (Example 1; Figure 1). Applicant teaches four different plant transformation vectors comprising regions encoding the leader peptide of DmAMP1, the mature protein of DmAMP1, a linker propeptide, and the mature protein domain of RsAFP2. The linker propeptide, which separates DmAMP1 and RsAFP2, is either from a Ib-AMP, a propeptide consisting of a part of DmAMP1 and a putative subtilisin-like protease processing site at its C-terminus, or a propeptide consisting of a part of AcAMP2 and a putative subtilisin-like protease processing site at its C-terminus (Example 2; Figures 3-6). A construct was also made for the expression of only DmAMP1 (Figure 7). Applicant also teaches transformation of Arabidopsis thaliana with said vectors and teaches analysis of the expression levels of DmAMP1 and RsAFP2 in the leaves (Examples 4-5; Table 1). The expression levels of DmAMP1 in plants transformed with the polyprotein (DmAMP1 and RsAFP2) vectors were much higher as compared to plants transformed for the expression of only DmAMP1 (Table 1; Figure 13). Further, analysis of proteins processed from the polyproteins revealed no

uncleaved fusion protein in the plant (Example 6). In addition, analysis of subcellular localization of the coexpressed proteins revealed extracellular localization of the polyproteins in all transgenic plants (Example 7 and Table 2).

Applicant has not disclosed a method that employs a fragment and variants (as defined in the specification) of the disclosed and non-disclosed linker propeptides, wherein the fragment or the variant acts as a propeptide and provide suitable cleavage sites. Applicant has not provided guidance for how to obtain all of the linker propeptides as recited in claims 4-12, and how to use them to achieve efficient expression and secretion of any desired proteins.

The specification, page 11, a "variant" of a linker propeptide is defined as sequences of amino acids which differ from the original sequence in one or more amino acids substitutions without altering the biological activity of the polypeptide. A "fragment" is defined, on page 13, as sequences from which amino acids have been deleted and that retain propeptide activity. However, Applicant has not provided guidance for modifications to the disclosed propeptides that resulted variants and fragments that retain propeptide activity.

The state of the art teaches unpredictability inherent in a protein function when one or more amino acids in that protein are modified. For example, Lazar et al (Molecular and Cellular Biology, March 1988, Vol. 8, No. 3, pp. 1247-1257 (U)) teach that a mutation of aspartic acid 47 and leucine 48 of a transforming growth factor alpha results in different biological activities (see at least the Title). Broun et al (Science, 13 November 1998, vol. 282, pp. 131-133 (U)) teach that as few as four amino acid

substitutions in a protein can change the protein activity (Abstract). Therefore, it is unpredictable whether any and all variants and fragments of the disclosed linker propeptides would retain the desired biological activity.

In addition, the working examples disclosed in the specification is limited to the expression of antimicrobial polyproteins separated by a linker propeptide from an antimicrobial protein. However, it is unclear whether the linker propeptide from the antimicrobial protein would also provide the same desired result with non-antimicrobial polyproteins.

Therefore, absent specific guidance for where to modify the desired linker propeptides so that the resultant variants and fragments would act as propeptide, one skilled in the art, who is willing to practice the invention, is left with trial and error experimentations considered to be undue.

Therefore, in view of the breadth of the claims, the limited guidance, and unpredictability inherent with respect to protein/polypeptide modifications, the claimed invention is not enabled throughout the broad scope.

See *Amgen Inc. Chugai Pharmaceutical Co. Ltd.*, 18 USPQ 2d 1016 at 1027 (Fed. Cir. 1991) where it is taught that the disclosure of a few gene sequences did not enable claims broadly drawn to any analog thereof.

Written Description

Claims 4-12 and 14 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as

to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The claims are broadly drawn to a method that employs any two or more proteins separated by a linker propeptide from a plant antimicrobial proteins of the genus Impatiens or a variant and fragment thereof s or a virus, and a chimeric propeptide comprising said variant and/or fragment, wherein said variant and fragment retain the propeptide activity.

The claimed invention does not meet the current written description requirement because Applicant has not described all proteins and linker propeptides required by the claimed methods. Applicant only describes a method that employs two plant defensins, namely, DmAMP1 and RsAFP2, separated by a linker propeptide from the antimicrobial proteins of Dm-AMP1 and AcAMP2. Applicant has not described other proteins and all variants and fragments of the linker propeptide as broadly claimed. Therefore, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that Applicants were in possession of the claimed invention.

See Written description Examination Guidelines published in Federal Registry/Vol. 66, No.4/Friday, January 5, 2001/Notices). See, also *University of California v. Eli Lilly and Co.* 43 USPQ2d 1398 (Fed. Cir. 1997).

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1-12, 14-16 and 18-21 are rejected under 35 U.S.C. 102(a) as being anticipated by Attenborough et al (WO 95/24486, Applicant's IDS)

The claims are broadly drawn to a method of improving expression of multiple proteins comprising inserting into the genome of said plant a DNA sequence comprising operably linked promoter region, a signal sequence, two or more protein encoding regions separated from each other by a linker propeptide coding sequence from plant microbial proteins including DmAMP1 and Ac-AMP2 or from virus and fragments and variants thereof, wherein the linker provides a post-translational cleavage site and wherein at least two of the protein encoding regions encode different proteins. The claims also encompass specific linker sequences, a hevein-type antimicrobial protein, and a subtilisin-like protease processing site.

Attenborough et al teach a method of expressing multiple proteins in a transgenic plant, said method comprising inserting into the genome of said plant a gene construct comprising a promoter operably linked to four Ib-AMP encoding sequences separated by linker or spacer propeptides from Ib-AMP which provides a cleavage site, whereby the expressed polyprotein is post- transitionally processed into the component protein molecules. The cited reference teaches that a signal sequence from Ib-AMP gene may be incorporated in the construct (pages 9-14), and that the linker propeptide can be obtained from a piconovirus (paragraph bridging pages 11 and 12). On page 14, first full paragraph, the reference disclosed that two or more sequences encoding different

proteins linked in one polyprotein can be expressed by using the same method. The cited reference teaches linker sequences (SEQ ID NO: 14-18) that fully anticipate the linker and the chimeric propeptides and variants and fragments thereof of claims 4-12, 14-15 and 19. In Examples 11-12, the cited reference teaches a DNA construct comprising a promoter, signal sequence, three copies of the antimicrobial protein Rs-AFP2 encoding sequences separated by propeptide linker sequences, for plant transformation, wherein the expressed polyprotein is subsequently processed to into three copies protein component. All claim limitations are disclosed by

Remarks

No claim is allowed.

Papers related to this application may be submitted to Technology Sector 1 by facsimile transmission. Papers should be faxed to Crystal Mall 1, Art Unit 1638, using fax number (703) 308-4242. All Technology Sector 1 fax machines are available to receive transmission 24 hrs/day, 7 days/wk. Please note that the faxing of such papers must conform with the Notice published in the Official Gazette, 1096 OG 30 (November 15, 1989).

Any inquiry concerning this communication or earlier communications from the Examiner should be directed to Medina A. Ibrahim whose telephone number is (703) 306-5822. The Examiner can normally be reached Monday-Thursday from 8:30AM to 5:30PM and every other Friday 9:00AM to 5:00PM.

If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, Dr. Amy Nelson, can be reached at (703) 306-3218.

Any inquiry of a general nature or relating to the status of this application should be directed to the receptionist whose telephone number is (703) 308-0196.

4/1/03
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